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Evaluating the safety and efficacy of placental antigen vaccines for fertility regulation

TASK FORCE ON IMMUNOLOGICAL METHODS FOR FERTILITY REGULATION*
WHO Special Programme of Research, Development and Research Training in Human Reproduction

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INTRODUCTION

Although substantial progress has been made over the last few years in the provision of family planning services to many of the World's populations, there is still an urgent need for a greater variety of safe, effective and acceptable methods of fertility regulation to meet widely differing personal, cultural and service requirements.

Immunization as a prophylactic measure is now so widely accepted that it has been suggested that one method of fertility regulation which might have wide appeal as well as great ease of service delivery would be an anti-fertility vaccine. It is essential, since they are to be used by healthy people, that all methods of fertility regulation be thoroughly tested from the point of view of safety. In the case of an anti-fertility vaccine, which represents a totally new area of intervention and whose effects are intentionally of long duration, this is even more important. This poses a major problem, since guidelines for the safety evaluation of fertility regulating vaccines do not exist. This document, which has been developed by the WHO Special Programme of Research, Development and Research Training in Human Reproduction, in consultation with immunologists, toxicologists, immunopathologists, reproductive biologists and three drug regulatory agencies, represents an attempt to define the parameters to be examined and the methodology which might be employed in such a safety evaluation.

It is not intended to be a definitive answer to what is a very complex problem. Rather, it is hoped that publication of this paper will encourage consideration of the safety criteria by the scientific community and result in the formulation of a definitive set of guidelines on which safety studies can be based.

Rationale

Evidence has been accumulated from work in animals and humans that there exist proteins specific to the reproductive system. If the action of physiologically active proteins could be blocked by immunological techniques, new methods of fertility regulation would become available.

In principle, anti-fertility vaccines may: (a) prevent sperm transport and/or fertilization; (b) prevent or disrupt implantation; and (c) prevent blastocyst development.

The potential advantages of an immunological approach to fertility regulation can be summarized as follows: (a) the possibility of infrequent administration, possibly by paramedical personnel; (b) the use of antigens or antigen fragments, which are not pharmacologically active; and (c) in the case of antigens of known chemical structure, there is the possibility of large-scale synthesis and manufacture of vaccine at relatively low cost.

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Antigens chosen as candidates for vaccine development should primarily be ones which do not share antigenic determinants with normal components in vaccine recipients. Otherwise, complications could result from interference with biological mechanisms or structures which are not the intended target. When there are common determinants, the required specificity may be obtained by identifying and synthesizing unique structures of the selected macromolecule.

In order to avoid damage to normal tissues by effects of the immune response, the natural antigen should: (i) not be present continuously in the vaccine recipients, and (ii) when present intermittently, it should occur in relatively low concentration. For fertility regulation in the female, these criteria are met by some placental hormones, by certain other macromolecules of the placenta and by sperm.

In some instances, antibodies primarily directed against placental antigens may show a low degree of cross-reactivity *in vitro* with some normal tissue constituent. If the degree of cross-reactivity is extremely low, it may nevertheless be justified to consider the placental antigen in question as a candidate for the development of a vaccine, subject to extensive *in vivo* assessment of all potential health hazards, as outlined below.

PART 1: EVALUATION OF EFFICACY AND SAFETY OF PLACENTAL ANTIGEN VACCINES

(1.0) *Description of material*

(1.1) *Antigens.* Placental antigens should not possess significant immunological similarity with any tissue other than placenta. Antigens without hormonal or other biological activity are preferable to biologically active compounds, since otherwise undesired side effects might occur. The selected antigen should be one that can be made available at a reasonable cost. If it is derived from normal placenta, methods to obtain it consistently in a highly purified form must be available. Should the selected antigen be a synthetic peptide, synthesis methods must be standardized to produce a uniform product in consecutive batches.

(1.2) *Carriers or haptens.* Many placental molecules require structural modifications in order to be antigenic or to overcome natural immunological tolerance. For larger molecular weight antigens, such modifications may involve coupling with smaller haptens; for small antigens such as peptides, conjugation to immunological protein carriers may be required. In order to be effective, the carrier or hapten must be foreign to the human. The antibodies produced against the coupled hapten or carrier should not be harmful and, if possible, beneficial.

(1.3) *Adjuvants.* In addition to chemical modification of the placental antigens, other procedures to enhance the immune response may also be required. An appropriate response may be achieved by administering the immunogen in an adjuvant. This procedure may not only increase the immune response in immunocompetent individuals, but may also overcome resistance to antibody formation in immunologically deficient persons, who normally would be poor responders.

Any adjuvant material should have minimal toxicity and should not produce unacceptable lesions at the site of injection. Toxicity of the selected adjuvant should be assessed separately from that of the immunogen; the ability of the adjuvant to enhance the immune response of genetically defined strains of animals should be examined. This is essential to deal with the expected problem of encountering non-responding individuals in genetically heterogeneous human populations.

(2.0) *Quality control procedures for production of vaccine components*

The following outline forms a proposal for the evaluation of antigens, adjuvants or other vaccine components synthesized or isolated for human use.

(2.1) *Purity.* Synthetic products to be used as antigens or adjuvants should be prepared by methods providing a reproducible degree of purity and yield. The purity of each batch should be assayed by total amino acid composition, determination of terminal amino acids and chromatographic analyses in three or more solvent systems. Sequence analysis should be performed on the initial preparation after production has begun and at intervals thereafter.

In the case of products derived from natural (biological) sources, each batch should be subjected to chromatographic or electrophoretic analysis. In addition, immunoelectrophoresis of each batch should be performed.

(2.2) *Safety evaluation.* Each batch of vaccine (in the form ready for human use) should be subjected to acute toxicity testing (see section 3.0). At least two species of laboratory animals should be injected with test materials and observed for 1 week for any ovbious signs of toxicity. All batches should be tested for pyrogenicity by standard assay methods.

(2.3) *Sterility.* Lots of final products, ampouled for distribution, should be tested for microbial contamination. The percentage of ampoules of each batch of vaccine tested will vary according to the nature of the product, but will probably be 0·1–0·5%.

(2.4) *Shelf-life.* Prior to distribution of any product subjected to the procedures described above, its stability for the purpose intended should be evaluated. Different groups of ampoules from the same batch should be tested for immunogenicity after being stored at different conditions of temperature and humidity for varying periods of time. The shelf-life of products under specific conditions can then be defined for subsequent lots.

(3.0) *Toxicity testing of materials*

Acute, subacute and chronic toxicity testing must be performed separately for the haptenated antigen or conjugate and adjuvant. The particular animal species to be used, testing procedures and length of observation required by drug regulatory agencies in different countries vary widely. Any vaccine developed will be shown to have sufficiently low toxicity and to be non-pyrogenic. It should be approved by the drug regulatory agency in the country where human trials are to be undertaken.

(3.1) *Animals.* Animal species differ widely in their response to drugs, and in the way in which the drugs are absorbed, distributed, metabolized and excreted. The mouse, rat, rabbit, dog and monkey are often used and their reactions are well documented. However, the evaluation of toxicity in higher primates should be encouraged in an attempt to select species whose reactions to vaccines are as similar as possible to those of man.

Healthy animals are required for toxicological studies, and animals from uncontrolled sources should be avoided. The drug response of animals is influenced by a variety of factors, such as diet, season, housing conditions, environmental temperature and humidity, the presence of infectious diseases and interactions of the drug with other substances. Impurities such as DDT, polychorinated biphenyls and nitrosamines have been found in some animal diets, and these may affect the response to a drug or to an antigen. Precautions should be taken to avoid unacceptable levels of such contaminants in animal diets.

Each study should include a sufficient number of animals per group to permit a valid estimation of the incidence and frequency of any toxic effects. The selection of group size for chronic studies will depend upon the toxicity data resulting from preliminary studies and other considerations, such as interim sacrifices, evaluation of reversibility of adverse effects and the assessment of other toxic effects, such as carcinogenesis. Experiments should be evaluated statistically and the numbers of animals used must be in accord with statistical requirements. It is recognized, however, that for a variety of purposes useful information may also be obtained from detailed experiments on a small number of animals.

(3.2) *Acute and sub-acute toxicity tests.* The purpose of acute toxicity studies is to define the range of lethal dosage following a single or a few closely-spaced administrations of the drug. These studies are part of the initial pharmacological screening programme in drug development. Detailed observations should be made on the effect of the drug upon important functions such as locomotion, respiration and behaviour. Obvious symptoms, such as convulsions and vomiting, should be looked for. These signs often furnish information about the cause of death; they may be supplemented by autopsy and if necessary, by histological examinations. The time-course of such events may vary considerably, depending on whether antigens or other components of the vaccine are considered. The use of animals of sex and age corresponding to the intended human use will provide insight into the rate of absorption and

metabolism of the compounds tested. The route, vehicle, volume and rate of administration should also be similar to those intended for human use.

In general, acute toxicity studies should be carried out in several species, including at least two rodent and one non-rodent species. These species should be, if possible, those likely to be used for long-term toxicity studies. The LD_{50} for rodents should be determined, if achieved with a reasonable multiple of the anticipated therapeutic dose, by standard statistical methods, such as probit analysis. For non-rodents, which are usually used in smaller numbers, an approximate determination should suffice.

For acute toxicity testing, animals of each species used should be injected both intramuscularly and intravenously. Three groups of animals, each receiving different doses, should receive a similar injection either intramuscularly or intravenously of the test compound. A control group should be tested in each species. Besides lethality, animals should be monitored for 1 week after administration of the drug, as outlined above, for changes in central nervous, respiratory and cardiovascular activities.

For subacute toxicity studies, similar doses to those used in the acute study will be administered at the rate of one injection per week for 4 weeks. Subcutaneous injections, instead of intravenous administration, will be performed. In addition to the overall assessment of toxicity as in the acute groups, subacute toxicity studies should include observations on body weight, food consumption, water consumption and additional evaluation of clinical signs. Animals should be observed for a period of 8 to 12 weeks following the initial administration. At the end of the period of observation, surviving animals should be killed and a detailed macroscopic examination performed. Organ weight should be determined, the tissue preserved and selected tissues processed further for histopathological examination.

(3.3) *Chronic toxicity testing.* The purpose of long-term toxicity studies is to provide information on the potential toxicity of a vaccine over a prolonged period of time, to assess whether reversible or irreversible damage occurs, and to determine which organ systems are involved. The duration of the chronic toxicity tests should be related to the expected duration of administration to man, as well as to the pharmacological actions and biotransformation of vaccine components. Whenever possible, such tests should be conducted in the species used for demonstrating efficacy of the vaccine and in which immunological hazards are most likely to occur.

Long-term toxicity tests should be conducted in at least two species of healthy, mature animals; one should be non-rodent. Studies should be performed at three different dose levels. Selection of the doses will be based upon the immunological and toxic properties of the total vaccine in the form intended for use. The lowest dose should be one that is believed efficacious in humans; the highest dose will be a reasonable multiple of the anticipated human dose. One intermediate dose should be included as well as appropriate control groups.

Vaccines should be administered to animals at intervals corresponding to the intended use in man. The same principle applies to the route of administration. The above-suggested intervals for subacute toxicity testing (4 weekly injections) should correspond to the maximum dose suggested for humans. Animals so treated will be maintained and killed after 3 months, 6 months and 1 year.

Following any animal deaths, autopsy shall be performed and an attempt made to establish the cause of death. In addition to the clinical observations outlined for short-term studies, the following haematological, blood chemistry and metabolic studies should be performed.

(3.3.1) *Haematology.* Before administration, and during weeks 4 and 13, blood samples should be examined for partial cell volume, red blood cells and white blood cells (total and differential). Initially, the study will be restricted to ten males and ten females from the control and high dosage groups, with subsequent extension to the intermediate and low dosage groups if some effects are shown at the highest level.

(3.3.2) *Blood chemistry.* Before administration and during weeks 4 and 13, blood samples will be examined for: urea, glucose, serum protein (total and differential), serum alkaline phosphatase (SAP), serum glutamic–pyruvic transaminase (SGPT), sodium and potassium. Samples will be drawn from five males and five females from each of the control and high dosage groups, in the first instance, with extension to other groups if indicated.

(3.3.3) *Urinalysis.* During weeks 4 and 13, individual overnight urine samples will be collected from

five males and five females from each of the control and high dosage groups, and examined for: pH, volume, glucose, ketones, specific gravity, bile pigments, protein, reducing substances, haemoglobin and urobilin.

Microscopy of urinary sediments will also be performed. Examination will be extended to other groups if indicated. Upon completion of clinical observations the following pathological evaluations should be made.

(3.3.4) *Gross pathological and organ weight analysis.* The macroscopic appearance of the tissues will be noted. The following organs from each animal will be dissected free of fat and weighed: heart, uterus and ovaries. Samples of the following tissues from each animal will be preserved: heart and heart valves, uterus, both lungs, gall bladder, thymus, liver, duodenum, spleen, tail of pancreas, caecum, kidney, eye, bladder, ovary, bone marrow, epididymis, pituitary, parotid and submandibular, mid-colon, trachea, tongue, sciatic nerve, testes, aorta, skin, skeletal muscle, jejunum, mammary gland, seminal vesicle, second eye, adrenals, prostate, lymph nodes (para-aortic), ileum, oesophagus, spinal cord, stomach (glandular and non-glandular) and brain (medullary, cerebellar and cortical sections), plus any other tissue showing macroscopic abnormality.

(3.3.5) *Microscopic evaluation.* Initially, histology will be restricted to: (a) abnormal tissues from animals that die, in an attempt to ascertain cause of death; and (b) all animals from the high dosage group, and eight males from the control group, killed at 13 weeks.

The study will then be extended to the intermediate and low dosage groups to cover any tissue showing some adverse effects at the highest dose level. The routine stain used will be haematoxylin and eosin. Other special histological and/or histochemical procedures will be employed as required for evaluation of selected tissues.

(3.3.6) *Ophthalmological tests.* For some antigens, such as synthetic peptides, standard ophthalmological tests (ophthalmoscopic evaluation) should be performed. The species used and the specific protocol will vary, depending upon the antigen involved, and should be determined for each vaccine tested.

(3.4) *Special studies.* Standard procedures of toxicity testing include the assessment of effects on reproduction and the evaluation of teratological effects. Although antibodies specific for human placental antigens are unlikely to have any direct effects on the foetus, certain components on the vaccine (e.g. carrier, adjuvant or vehicle) may be toxic for developing embryos. In order to ensure the safety of vaccines given inadvertently to pregnant women, injections of vaccines to pregnant animals should be performed and term foetuses examined carefully for anomalies. Specific protocols should be developed for each species of animal used.

Vaccines used against pathogenic micro-organisms are usually designed to provide immunity for a long time; variations in the time at which immunity may be lost are usually of little concern. However, vaccines designed to inhibit or disrupt fertility should be carefully evaluated for reversibility and the return of vaccinated subjects to a fertile state. Studies in animals and in phase I and II human trials should be performed in sufficient depth to indicate whether the method used has permanent or reversible effects. If the effect is reversible, the range of time elapsed since the last immunization until fertility is restored should be evaluated. It should be assessed also whether intermittent contact with the natural placental antigen and/or low levels of cross-reactivity with normal body constituents will have any boosting effect on the immune response achieved by active immunization. Specific protocols should be developed for each vaccine developed.

(4.0) *Animal models for efficacy and immunological safety evaluation*

(4.1) *Anti-fertility testing.* In order to evaluate in an animal model the effectiveness of a human, natural or synthetic antigen, as part of a fertility regulating vaccine, tests must be performed in a species where an analogous antigen exists and possesses extensive immunological similarity with the human material. This requirement clearly makes the use of subhuman primates for testing placental antigens mandatory. In species of subhuman primates where immunological cross-reactivity of the endogenous antigen with its human analogue is low, the animal placental antigen can be used as a model providing its

physiological function is the same as that of the human analogue. Both human and animal antigens should be placenta-specific.

The use of animal placental antigens as models may permit the use of lower evolutionary species of primates, such as monkeys; however, direct testing of human placental antigens in animals will likely require baboons, gorillas or chimpanzees. The validity of efficacy evaluations depends heavily upon the immunological similarity of exogenously prepared antigens and endogenous functional substances in the test animal.

(4.2) *Immunological safety evaluation.* Essentially the same requirements apply for evaluating the immunopathological safety of the vaccine as for testing anti-fertility efficacy. When human antigens are tested in animals, the reactivity of antibodies produced in the animal with endogenous placental or maternal substances should be precisely the same as that expected to occur in humans. This reactivity can be predicted by *in vitro* tests of cross-reactions, as outlined in section 5.0. For testing human antigens, the use on non-human primates is therefore essential for safety evaluation. The selection of a particular primate for safety testing will depend upon the antigenic similarity between the human antigen used in the vaccine and the endogenous primate analogue. For the testing of human placental antigen vaccines, only baboons, chimpanzees and gorillas are believed to be suitable models. Sufficient numbers of these animals must be studied to provide confidence in the results of the tests.

In addition to the immunological safety and efficacy evaluations, a selection of the most appropriate conjugates and adjuvants for widespread use in an outbred population should be made by studying the immune response to the vaccine in different and genetically defined animal strains. This requirement precludes non-human primates, but can readily be met in rodents. These studies will precede any evaluation of safety and efficacy in primates.

Animals used for toxicity testing are discussed in detail in section 3.0.

(5.0) *Monitoring of the immune response and potential hazards of immunization*

The studies performed until now in experimental animals have clearly indicated that animals immunized with some placental antigens in the form of haptenized proteins or peptide–protein conjugates develop both a humoral and cell-mediated immune response to various components of the immunogenic conjugate. This response should be monitored in quantitative and qualitative terms, both for the humoral (section 5.1) and for the cell-mediated (section 5.2) immune responses.

Immunization always entails some potential risks. Some risks are inherent to any repeated immunization with foreign proteins. Evaluation for this type of risk and proposals for safety studies in this respect are dealt with in section 5.3. Other risks such as autoimmune (5.4) and immune complex disease (5.5) are more specifically related to immunization with placental antigens.

(5.1) *Monitoring of humoral immune responses.* The following tests should be performed in immunized animals: (a) direct antibody binding of homologous antigens (native protein such as placental hormone or other substance; carrier protein and whole conjugate used as immunogen); (b) antibody neutralization of physiological activity of antigen; and (c) skin tests for antibody-dependent reactions (anaphylactic and Arthus).

(5.2) *Monitoring of cell-mediated immune responses.* The following tests should be performed in immunized animals: (a) skin-testing for delayed hypersensitivity (DTH); (b) macrophage migration inhibition assay (MIF); and (c) lymphocyte blastogenic test (LBT).

The antigens used for the tests monitoring the cell-mediated immune response will be the same as those indicated in 5.1 for monitoring humoral immune responses.

(5.3) *Hazards of repeated immunizations.* Although the practice of vaccination with toxoids and other vaccines indicates that local allergic reactions seldom represent a problem, it must be considered that the anti-fertility vaccine may have to be administered more frequently in order to maintain an immune response level adequate for neutralization of antigen. Accordingly, the hazards could become more like those encountered by allergists when repeated injections of allergens are performed. Among the potential hazards of repeated immunizations are those listed below.

(5.3.1) *Localized inflammatory reactions.* Such reactions may be seen at the site of injection. Evalua-

tion of skin tests in immunized animals (5.1.c, 5.2) should provide information about the incidence and severity of local skin reactions to be expected in immunized women.

(5.3.2) *Generalized allergic reactions.* Anaphylactic reactions are the ones to be feared. Although the occurrence of generalized anaphylactic reactions to the repeated intramuscular or subcutaneous injection of doses of foreign proteins in the range of 100–200 μg represents a definite hazard, the magnitude of this hazard will only be appreciated at the stage of extensive human trials. Physicians practising desensitization therapy with allergens encounter a rather low incidence of generalized allergic reactions; in a normal population, the incidence would be even lower. Recent data indicate that the population of allergic patients with atopic diseases such as asthma or hay fever is not at greater risk of developing generalized allergic reactions to injected foreign proteins than the non-allergic population. Furthermore, immunization of the intended type will induce IgG and IgM antibodies rather than the IgE antibodies responsible for anaphylactic reactions. In order to assess the potential development of anaphylactic hypersensitivity with the immunization procedure used, the following tests should be performed in immunized animals or with their serum: (1) antigen-specific IgE antibody levels (RAST test); (2) Prausnitz-Kustner tests in animals; and (3) skin testing for anaphylaxis and Arthus reactions (as 5.1.c).

(5.4) *Autoimmune disease.* It is theoretically possible that repeated injections of an antigen from a naturally occurring product of the body, particularly if conjugated to a foreign carrier, could induce autoimmunity affecting various tissues. This is particularly important when placental antigens, such as hormone antigens or antigenic fragments thereof, are used to produce antibodies against the native hormone, unless there is absolute certainty that the substance used in the vaccine is not normally produced by any cells of the body except those of the placenta. In the case of placental antigens, careful studies should be performed to preclude their production by normal cells of the non-pregnant woman. The presence of autoantibodies, e.g. antibodies to thyroid, pituitary, stomach, adrenal hypothalmus, ovary, uterus, bone marrow, intestine, pancreas, liver, skin, kidney, lung and myocardium, should be tested for following immunization. In addition, anti-organelle antibodies, including anti-nuclear antibody, rheumatoid factor, anti-smooth muscle and anti-mitochondrial antibodies, should be looked for. In order to assess autoantibodies, the following tests must be performed on immunized animals: (1) direct antibody binding of soluble, potentially cross-reacting, antigens (various hormones and other purified antigens); (2) monitoring of serum levels of reproductive hormones in immunized animals; (3) neutralization by the antibodies produced of the biological activity of suspected cross-reacting substances; (4) indirect immunofluorescence (IF) studies on sections of normal animal tissues; (5) complement fixation (CF) tests on normal tissue extracts; and (6) latex agglutination for rheumatoid factor.

(5.5) *Hazard of immune complex disease.* The production of high titre antibodies to antigen determinants, which are probably needed for physiological neutralization *in vivo,* will lead to the formation of immune complexes under various circumstances: (a) upon boosting re-injection of the vaccine; (b) upon early endogenous production of antigen in the case of conception occurring in already immunized women; (c) upon immunization of already pregnant women, possibly immunized during the early undetected period of pregnancy; and (d) upon cross-reaction of antibodies with endogenous maternal antigens in the blood.

Immune complex formation induced locally upon re-injection of the vaccine is not expected to cause problems more serious than those encountered upon repeated vaccination or desensitization therapy. If large amounts of circulating immune complexes were produced, the development of acute glomerulonephritis analogous to that encountered in serum sickness becomes a possibility. This could occur if immunization were performed during undetected pregnancy or if pregnancy occurred in a poorly immunized woman. Under such circumstances the formation of damaging immune complexes in slight antigen excess might be favoured.

Another potential source of kidney-damaging immune complexes would be the presence of antibodies cross-reacting with endogenous maternal antigens, especially if the neutralization of these antigens led to a compensatory hypersecretion or production of antigen. This is very unlikely, but should nevertheless be evaluated.

In any case, it is essential that the animals and women immunized with placental antigens are carefully

monitored for the appearance of circulating immune complexes and signs of immune complex-mediated tissue injuries (including glomerulonephritis). Women developing a poor antibody response would be especially at risk of developing immune complex disease.

In order to evaluate the existence and potential damage caused by immune complexes in immunized animals, the following tests must be performed.

(5.5.1) *Circulating immune complexes in sera.* The currently available assays are not specific for circulating immune complexes, but detect essentially aggregated IgG. They may be interfered with by various substances, including DNA, endotoxins and polyanions (e.g. heparin). Among numerous assays recently compared, the Raji cell radioimmunoassay (IRCA), the C1q binding assay and the C1q solid phase (C1qSPA) assay appeared to offer the best correlations with the presence of immune complexes.

For screening circulating immune complexes in immunized animals, the following assays should be used: (a) C1qSPA; (b) IRCA; and (c) radial immunodiffusion for C3 and components of C3.

On positive serum samples, the immune complex nature of the aggregated material should be established by determination of the molecular size of the material (by sucrose gradient ultracentrifugation) and by the identification, in the complexes, of the antigens used in the immunization.

(5.5.2) *Detection of immune complex deposits and tissue injury.* There is now ample evidence that circulating immune complexes are not always associated with tissue damage. Definite evidence of immune complex-mediated tissue injury requires the direct demonstration of immune complexes deposited in the tissue and causing damage. For this purpose, serial kidney biopsies should be performed and a more extensive search for immune complexes in other tissues should be made at autopsy. This will involve: (a) immunofluorescent analyses of kidney biopsies (for globulin and complement), and at autopsy immunofluorescent analyses of kidney, heart valves, spleen, choroid plexus and possibly other tissues; (b) histopathological evaluation of renal biopsies and other tissues at autopsy; and (c) urine analysis for protein.

(6.0) *Determination of anti-fertility efficacy*

Once immunity against the reproductive antigen has been established in a statistically sufficient number of non-human primate animals, repeated mating of females should be performed in order to assess fertility reduction. The potential fertility of the females should be determined (e.g. ovulatory level of progesterone in the second half of the cycle), and variations in menstrual cycle parameters observed. Fertility rates of non-immunized animals within the same colony should be known. Only males of proven fertility should be used in these tests. Control animals receiving adjuvant and carrier only, and non-treated animals, should also be mated in parallel to confirm fertility of the animals during the same period as the immunized subjects. Methods for detecting and monitoring pregnancy must be available to accurately evaluate efficacy.

(7.0) *Evaluation of data and criteria for determination of vaccine safety*

This document should provide a general outline of the steps required for the successful development of vaccines for human fertility regulation. Specific protocols should be prepared for each individual vaccine under consideration, since some aspects of safety evaluation will need more emphasis in one case than in the other. For some of the safety tests, positive results would directly indicate a health hazard, but this is not necessarily true for all tests used (e.g. isolated immunofluorescence). Below are listed the criteria that, in general, would contraindicate use of a vaccine.

(1) The neutralization or inhibition of the biological functions of any substance other than the target antigen of the vaccine.

(2) Clear demonstration of antibody binding to normal non-target tissues that persists long after the initial immunization and/or that results in manifest cytotoxicity.

(3) Persistent intravascular immune complexes which result in complex deposits in the kidney provoking pathological damage.

(4) Any significant number of observations of anaphylactic reactions to repeated immunization.

(5) Evidence of teratological effects of immunization on offspring of mothers immunized during pregnancy or on offspring born after immunity has waned.

PART 2: EVALUATING THE SAFETY AND EFFICACY OF AN HCG-PEPTIDE VACCINE

(1.0) *Rationale*

The advantage of immunizing against a pregnancy-specific placental hormone, such as human chorionic gonadotrophin (HCG), is that the antigen is probably present in immunized women only at times of incipient pregnancy, and that risks of side effects would be thereby considerably minimized. There is still some question, however, about the potential disadvantage of immunization against whole human chorionic gonadotropin, in that the α subunit of CG is antigenically and structurally the same as that of other pituitary hormones, such as FSH, LH and TSH. Furthermore, the β subunit of HCG, and presumably of all chorionic gonadotropins of primate species, has marked chemical similarities and cross-reactivity with pituitary LH. This cross-reactivity might be a cause of undesirable immunological side effects in immunized women. On the other hand, the C-terminal fragment of β HCG, comprising thirty-three amino acid residues, is chemically distinct from the C-terminal fragment of the β subunit of HLH or of any other known polypeptide hormone. Antibodies to this terminal fragment of the HCG β subunit neutralize the biological activity of HCG without having any detectable cross-reaction with subunits or intact polypeptide hormones other than HCG. For that reason, an anti-fertility vaccine based upon immunologically active conjugates of peptide sequences unique to the β subunit of the HCG molecule may be developed.

(2.0) *Intended method of use*

It is intended to immunize women by injection of the peptide conjugate in adjuvant. Testing in baboons and chimpanzees and phase I and II trials will reveal whether a single injection is sufficient to achieve the desired level of immunization, or whether several boosting injections will be required. The main desired effect is to achieve a degree of immunization sufficient to: (a) neutralize the hormonal activity of HCG *in vivo*; and (b) prevent or disrupt implantation at a very early stage of pregnancy.

Undesirable potential effects of the intended immunization are: (a) cross-reactivity of the antibodies or of sensitized lymphocytes with pituitary hormones, leading to biological neutralization of these hormones *in vivo* and/or damage to secretory cells; (b) formation of antibodies cross-reacting with any normal tissue antigen of the immunized host; (c) formation of persistent immune complexes and deposition of such complexes in tissues, especially the kidney; (d) teratological effects on progeny of women with poor immune response, and failure of immunization; and (e) frequent occurrence of sizeable local or generalized allergic reactions.

The main purpose of the projected experimental studies in non-human primates is to assess whether the undesired potential effects listed above indeed occur, or merely represent theoretical risks which are not realized in practice. A detailed discussion of the potential hazards involved have been given in section 5 of Part 1 of this document.

It is not yet established whether immunization with the β HCG peptide conjugate will cause an irreversible biological neutralization of HCG, or whether the immunity level, without further boosting, will sink until an inefficient level is reached after a period of time. This will probably vary from individual to individual. In the first case, the indication for immunization will be restricted to sterilization, whereas in the second eventuality, which on the basis of present information appears more likely, immunization may be considered as a long-lasting but reversible anti-fertility measure.

(3.0) *Protocols for safety studies to be completed before initiation of phase I human trials*

(3.1) *Experimental animals.* The following groups of animals will be immunized and will be tested as outlined in later sections.

(3.1.1) *Minimally immunized baboons.* Twenty adult female animals will be immunized according to

the standard protocol expected in the human trials, which will represent the minimum immunization needed to produce 100% anti-fertility. The primary injection of peptide–carrier adjuvant will be given intramuscularly at two sites in the pelvic region. A second immunization will be given 4 weeks later. Booster injections will be given as required to maintain the desired level of antibody over an extended period of time.

(3.1.2) *Hyperimmunized baboons.* Ten adult female baboons will receive the same dose of peptide carrier, but at 3 week intervals, following the primary immunization, for a total of five injections. Booster injections will be given as required to maintain a high level of antibody over an extended period of time. The same schedule will be used for six control animals receiving the adjuvant mixture only and six animals receiving adjuvant and carrier. Six baboons receiving no treatment will also be used as controls.

(3.1.3) *Hyperimmunized chimpanzees.* Eight adult female chimpanzees will be immunized with the vaccine according to the schedule outlined for hyperimmunized baboons. Since evaluations of vaccine safety will be limited to endocrinological function studies in these animals, each animal will serve as its own control and normal values of serum components established before immunization.

(3.2) *Monitoring of the immune response in immunized animals.* Serum and blood samples will be obtained prior to immunization to serve as antibody and cell controls for each animal. Subsequently, 10–12 ml will be obtained weekly throughout the period of observation for the listed tests. Every fourth week, an additional 20 ml of blood will be taken for the *in vitro* assays of cell-mediated immunity. Blood sampling will be done in each menstrual cycle in order to determine hormone levels and to confirm that ovulation has occurred. The tests indicated in Table 1 shall be performed on blood samples from all baboons and those listed in objectives (1) and (3) of Table 1 will also be carried out on chimpanzee blood samples.

(3.3) *Evaluation of potential effects of immunization with HCG conjugates on function and secretory tissues of other hormones.* Several potential complications have to be considered upon immunization of humans with immunogenic conjugates of HCG-derived antigens. Some of these hazards are related to potential disruption of the endocrinological balance and maternal–embryonal relationships; others are related to the possible autoimmunity induced with human-derived material as an autoantigen. Consideration of these potential hazards should provide guidelines for design and evaluation of animal and human studies.

(3.3.1) *Effects of hormone function.* It is unlikely that a vaccine prepared on the basis of the entire β HCG subunit could be able to induce an immune response entirely devoid of cross-reactivity with FSH, LH or TSH antigenic determinants. As a consequence, the possibility that immunization with β HCG subunit might cause neutralization of FSH, LH and TSH *in vivo* has to be considered as a serious potential hazard. Consequences of the neutralization of some of the pituitary hormones in females of reproductive age may be interference with thyroid or ovarian function, menstrual disturbances and possibly compensatory hyperfunction of the pituitary. For this reason, immunization with synthetic peptides entirely devoid of cross-reactivity appears to be a much safer approach. In this way, cross-reaction with pituitary hormones can probably be avoided. Even so, there is, at least theoretically, a mechanism by which immunization with HCG-specific peptides might lead to the production of auto-antibodies against pituitary hormones by immunological escalation: determinants of the β HCG C-terminal peptide, against which an immune response has been induced by repeated immunization, could function as carrier determinants and ultimately might elicit a response against determinants on the β subunit in common with LH or other common determinants on the α subunit. Although such a possibility appears remote and has no natural precedent, it should be evaluated, especially after long-term immunization. Studies on cross-reactivity should, therefore, also be performed with antisera collected long after the initial immunization, since the specificity and avidity of the antibodies induced may change with time.

Since the most likely substance that would cross-react with antibodies to HCG peptides are the glycoprotein hormones secreted by the anterior pituitary gland (FSH, LH and TSH), the serum levels and patterns of these hormones should be carefully studied before and after immunization in both baboons and chimpanzees. Other pituitary hormones, such as prolactin and growth hormone, should also be measured. In addition to the direct measurement of these hormones in the serum, the levels of

certain hormones in target organs should be monitored. Of particular importance will be thyroid and ovarian hormone secretion. For the thyroid evaluation, levels of thyroxine (T_4) and of tri-iodothyrinine (T_3) binding will be measured. For assessment of the ovarian function, levels of oestrogens and progesterone will be determined. Adrenal function will be monitored by measurement of plasma cortisol.

Should any data suggest that any endocrine function is impaired, standard stimulation tests will be used to determine whether the pituitary will respond to stimuli in a normal fashion relative to thyroid, adrenal and ovarian trophic hormone secretion.

(3.3.2) *Tissue damage.* The damage caused to the pituitary by antibodies or sensitized lymphocytes specific for antigenic determinants common to HCG and LH must theoretically be of major concern. The fact that immunization will be against a portion of the β HCG subunit unique to that molecule renders cross-reactions with pituitary hormones and ensuing pituitary damage rather unlikely. Such damage could occur by several immunological mechanisms, such as direct complement-dependent antibody cytotoxicity, antibody-dependent cell-mediated cytotoxicity, or cell-mediated immunity. Immunological reactions within the pituitary between hormone-specific antibodies or lymphocytes, on the one hand, and hormone-producing cells, on the other, could conceivably lead to pathological changes in the adenohyphophysis.

In addition to potential damage to pituitary cells, a theoretical hazard to thyroid tissue exists. It is known that human thyroid cells have a low number of HCG receptors which explains why thyroid function increases slightly during normal pregnancy. Should a pregnancy be established prior to immunization, antibodies could react with HCG on the thyroid cells and the resulting complexes result in tissue damage. Whether this would occur depends upon many variables which are rather unlikely but, nevertheless, careful evaluation of thyroid tissues after immunization should be performed.

The other tissues to be studied and the procedures to be used are described in Part I (3.3 and 5.4).

(3.4) *Retarded clearance of antibody in non-immunized animals.* Highly purified baboon IgG, specific for the HCG peptide, will be radiolabelled with ^{125}I and injected intravenously into normal baboons. This is an *in vivo* model to check *in vitro* tests for the possible cross-reactions of anti-conjugate antibodies with normal baboon components. Blood plasma will be monitored in these baboons for the disappearance of this radioactive purified baboon IgG. This will be compared with the clearance of an ^{131}I-labelled baboon IgG of different specificity (anti-bovine serum albumin) in two additional baboons. The baboons will be pre-treated with iodine to block the thyroid uptake. Blood samples will be collected at 30 min, 1, 4 and 24 hr, then daily until 50% of the radioactive tracer has been eliminated from the bloodstream.

(3.5) *Duration of the study before initiation of phase I trials in humans.* It is proposed that these studies should have been conducted for a minimum of 6 months prior to initiation of phase I trials in humans.

(4.0) *Protocols to be completed before initiation of phase II human trials*

(4.1) *Long-term toxicity and pathology.* The total of thirty baboons in the minimally and hyperimmunized groups should be studied for 5 years. Interim killing will be conducted at intervals of 2 and 3 years. Four animals from the minimally immunized group and two from the hyperimmunized group will be killed on each of these two occasions. The remaining twelve and six animals in the minimally and hyperimmunized groups respectively will be killed at the end of the 5 year study. At each autopsy, histopathological examinations will be made, with special reference to the reproductive organs, pituitary, thyroid, adrenal, kidney, spleen, liver and brain. Special consideration will be given to tissue involved in immune reactions, as indicated by other tests. Renal biopsies will be taken in the minimally immunized and hyperimmunized groups at 2 years and 4 years from the start of immunization. Immunofluorescent techniques will be employed to determine whether immune complexes have been deposited. Light and electron microscopy will be used to determine whether tissue damage has been caused. In selected samples, attempts will be made to elute immune complexes from renal biopsy specimens for the identification of (a) the specificity of the antibody and (b) the corresponding antigen. The control group of animals receiving carrier and adjuvant only will be studied according to the same protocol. It is not considered that these ongoing histopathological studies should be completed before initiation of phase II trials.

(4.2) *Evaluation of effects of immunization during pregnancy.* Once human immunization has started in a

TABLE 1. Tests to be performed in primates prior to initiation of human trials

(a) Prior to phase I human trials

	Objective	Animal groups to be tested	Nature of test	Timing, duration and frequency of test
1	Monitoring the humoral immune response	Minimally immunized baboons Maximally immunized baboons No treatment control baboons Adjuvant only control baboons Adjuvant and carrier control baboons Maximally immunized chimpanzees	(a) Direct antibody-binding of homologous antigens (hCG conjugates, etc.) (b) Neutralization of hCG by antibody	(a) 3, 5 and 7 weeks after immunization, monthly thereafter (b) 3, 5 and 7 weeks after immunization, monthly thereafter
2	Monitoring the cellular immune response	Minimally immunized baboons Maximally immunized baboons No treatment control baboons Adjuvant only control baboons	(a) Skin test for DTH (b) Macrophage migration inhibition (MIF) (c) Lymphocyte blastogenic test (LBT)	(a) Twice within first 6 months after immunization (b) 3 weeks after immunization (c) 3 weeks after immunization
3	Evaluating the risk of cross-reactions and autoimmunity	Minimally immunized baboons Maximally immunized baboons No treatment control baboons Adjuvant only control baboons Adjuvant and carrier control baboons Maximally immunized chimpanzees	(a) Direct antibody binding of heterologous antigens (hormones and other purified antigens) (b) Serum levels of hormones (LH, etc.) (c) Antibody neutralization of biological activity of other hormones (d) Immunofluorescence (IF) of sections of normal baboon tissue (e) Complement fixation (CF) with normal baboon tissue extracts	(a) Peak titre and after 6 months post-immunization (b) Peak titre and after 6 months post-immunization (c) Peak titre and after 6 months post-immunization (d) Peak titre and after 6 months post-immunization (e) Peak titre and after 6 months post-immunization

TABLE 1 (contd.)

(a) Prior to phase I human trials (contd.)

Objective	Animals groups to be tested	Nature of test	Timing, duration and frequency of test
4 Evaluating the risk of immune-complex disease	Minimally immunized baboons Maximally immunized baboons No treatment control baboons Adjuvant only control baboons Adjuvant and carrier control baboons	(a) Detection of immune complex in serum: (i) Clq solid phase RIA (ii) Raji cell assay (iii) Radial immunodiffusion for C3, C4	(a) 2 and 7 days after each immunization and after 6 months
		(b) Detect antigen and molecular size of circulating immune complex	(b) Each 6 months after immunization
		(c) Detection of immune complex deposits and kidney injury: (i) IF analysis of kidney biopsies (or autopsy) for globulin and complement (ii) Histopathological evaluation of kidney biopsy (or autopsy) (iii) Urine analysis	(c) Weekly during first 6 weeks of immunization
5 Evaluating the risk of anaphylactic hypersensitivity	Minimally immunized baboons Maximally immunized baboons No treatment control baboons Adjuvant only control baboons Adjuvant and carrier control baboons	(a) Anti-hCG IgE antibody levels (RAST) (b) PK test in baboons (c) Skin-testing for anaphylaxis and Arthus reaction	(a) Peak titre and at 6 months after immunization (b) Peak titre and at 6 months after immunization (c) Peak titre and at 6 months after immunization
6 Evaluating the risk of cross-reaction and autoimmunity	Normal baboons passively administered anti-hCG peptide antibody	(a) Passive administration of ^{125}I-labelled purified anti-hCG antibody; follow-up of elimination	

TABLE 1 (contd.)

(b) Prior to phase II human trials

	Objective	Animal groups to be tested	Nature of test	Timing, duration and frequency of test
7	To continue evaluation of long-term risks (3) (autoimmunity) and (4) (immune complex disease)	Minimally immunized baboons Maximally immunized baboons	(a) Continuation of tests 3a to 3e and 4a to 4c (b) Ongoing histopathological studies	(a) 2, 3 and 5 years after start of immunization (b) 2 and 4 years after start of immunization
8	To evaluate the risk of immune complex disease in pregnancy	Pregnant baboons (immunized after pregnancy confirmed)	(a) Tests 4a to 4c	(a) Throughout pregnancy and after abortion or term delivery
9	To evaluate teratological effects of immunization on progeny	Pregnant baboons (immunized during confirmed pregnancy). Baboons mated soon after first immunization. Baboons mated during waning immunity	(a) Histopathological examination of aborted foetuses (b) Medical examination and follow-up of delivered progeny	(a) Whenever these occur (b) At least 5 years *post-partum*

(c) Prior to phase III human trials

	Objective	Animal groups to be tested	Nature of test	Timing, duration and frequency of test
10	To evaluate the risks of autoimmunity and immune complex disease	Minimally immunized baboons Maximally immunized baboons	(a) Histopathological examination after prolonged immunization	(a) At least 3 years after immunization

phase I clinical trial involving women not capable of becoming pregnant, studies should be initiated on another group of animals to study the effects of (a) immunization in pregnant baboons, and (b) possible teratological complications.

(4.2.1) *Immunization of pregnant baboons.* Immunization by the standard procedure will be performed in pregnant baboons, but will start only when pregnancy has been confirmed. Because of the uncertainty of the success of immunization with the vaccine in the presence of serum CG, weekly blood samples will be assayed for normal hormone patterns associated with baboon pregnancy, and, in addition, the following assays will be performed: CG level, anti-conjugate antibody titre, detection of immune complexes and urine analyses for protein. Regardless of whether proteinuria is found, renal biopsies will be carried out to look for tissue-bound immune complexes and pathological changes. Five animals will be studied in this group.

(4.2.2) *Teratological studies.* In addition to women being immunized inadvertently during an established pregnancy, foetuses could be exposed to potential teratological effects of immunization in cases where sufficient antibody levels had not been attained prior to a successful mating or where antibody levels had waned since the last injection. The latter situation will certainly occur if the effect of the vaccine is reversible and if women intend to conceive following a period of induced infertility. In order to assure that the offspring produced in these situations are normal, animal studies will be conducted to intentionally obtain pregnancies during low antibody levels.

Five female baboons will be mated 2 weeks after their primary immunization. At this time, minimum antibody levels would be attained and conception should probably occur. If conceptions do not occur, another group of five animals will be mated 1 week after immunization. Pregnancies will be allowed to continue to term and the health status of mothers during pregnancy carefully monitored. Following the birth of the infant, a careful physical examination will be performed. All young baboons will be kept until they are adults (at least 5 years). During this time, various tests of physiological function will be carried out, particularly endocrine function. After attaining maturity, the ability of each animal to reproduce will be tested and the normality of their offspring evaluated.

Five other female baboons will be mated after antibody levels have waned following a standard immunization. When antibody levels suspected of providing marginal anti-fertility effects are observed, mating will begin and will be continued until conception occurs. Monitoring of mothers and evaluation of offspring will be conducted, as described above, for animals mated soon after primary immunization.

(4.3) *Evaluation of effects of hormone injections on immunized animals.* Allergic responses (anaphylaxis, Arthus, delayed hypersensitivity and serum immune complexes) may theoretically occur in immunized animals, not only from the interaction of injected antigen, but also from naturally formed CG produced by the trophoblast. The latter situation could occur if: (a) the initial CG production by the trophoblast before implantation were sufficient to produce a local reaction or (b) the pregnancy occurred despite immunization; e.g. due to the waning or deficiency of the anti-fertility immunity. A group of ten immunized baboons will serve as *in vivo* models to test for such reactions to natural (purified) hormones. The ten baboons will receive the standard immunization regime of two injections a month apart, and the studies will commence after the maximum titres of antibodies have been reached. Three individual animals will receive, intravenously, 50, 10 and 1·0 μg HCG respectively. Sera will be collected for immune complex assessment at 30 min, and 1, 4 and 24 hr. Urine abnormalities and renal biopsies for glomerular immune complexes and histopathology will be determined in these animals. Two additional animals will be skin-tested using 5·0 and 50 μg of hormone.

To ensure that anti-HCG conjugate antibodies show no cross-reaction with LH, the remaining five baboons will be tested with HLH (as described above for HCG). Finally, a control group of five baboons will receive the carrier–adjuvant mixture alone.

(5.0) *Protocols to be completed prior to phase III human trials*

Phase III human trials could be initiated if the baboon studies discussed above and phase I and phase II human trials have revealed no unacceptable side effects. Histopathological examination of animals

immunized for a minimum period of 3 years would, however, be required prior to this phase III evaluation.

(6.0) *Testing the anti-fertility efficacy*

Both minimally immunized and hyperimmunized baboons, as well as control animals receiving carrier and adjuvant only, will be mated with males of proven fertility. The immunized animals will be mated after significant antibody levels have been obtained (usually 6–8 weeks), and after it has been confirmed they have remained ovulatory, as indicated by the luteal phase elevations of serum progesterone.

Menstrual histories and events of the cycle will be defined in all animals prior to mating. Females will be cohabitated with males, beginning 3 days prior to the anticipated ovulation, and removed from the mating cage when sex skin deturgescence is observed (about 2 days post-ovulation). Serum samples will be collected every 48 hr following separation from the male, and pregnancy testing will begin on the calculated day 12 of gestation. Pregnant animals will be bled twice weekly to monitor hormone levels, antibody titres and other immunological parameters. Baboons not conceiving (or not remaining pregnant past the expected menses) will be evaluated for evidence of ovulation in the mating cycle. Ovulatory animals which are not pregnant will be mated again in the next cycle. This procedure will be repeated for at least three consecutive cycles.

Effectiveness of immunizations for preventing or disrupting gestation will be evaluated by two separate criteria. The first will be a tabulation of the proportion of animals that attained a sufficient antibody level following immunization, and the second will be the proportion of baboons attaining sufficient antibody levels that remained pregnant for more than three weeks past the expected menses. Should pregnant animals abort at various times during pregnancy, these times will be tabulated and the aborted foetuses examined carefully for anomalies. The health status of mothers following any abortions will also be assessed.

Sera from pregnant animals with significant antibody levels to HCG will be carefully assessed. Cross-reactivity of anti-HCG sera with baboon CG will be tested both *in vitro* and *in vivo* (bioassay).

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